Genomic Medicine, Biomarkers, and Health Sciences (2012) 4, 16—18

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.e-gmbhs.com

SHORT COMMUNICATION

Significance of migration-related genes (*S100A9*, *MAGED4*, *C8orf30A*, *IL-8*) in esophageal squamous cell carcinoma

Ching-Tang Huang [a], Guan-Cheng Huang [b], Pey-Shan Wu [c], Li-Wha Wu [d], Siao-Han Lin [a], Wu-Wei Lai [e], Yi-Ching Wang [f], Hsiao-Sheng Liu [a,c,*]

[a] *Institute of Basic Medical sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[b] *Division of Hemato-oncology, Department of Internal Medicine, Yuan's General Hospital, Kaohsiung, Taiwan*
[c] *Department of Microbiology & Immunology, National Cheng Kung University, Tainan, Taiwan*
[d] *Institute of Molecular Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan*
[e] *Department of Surgery, College of Medicine, National Cheng Kung University Hospital, Tainan, Taiwan*
[f] *Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

Received 11 March 2012; accepted 31 March 2012
Available online 8 May 2012

KEYWORDS
biomarker;
ESCC;
metastasis

Abstract To identify any biomarkers related to the migration of esophageal squamous cell carcinoma (ESCC) cells, ESCC CE81T cells were used to establish the CE81T-1 subline, which demonstrates increased migration activity after Transwell screening and microarray analysis. Among the differentially expressed genes, *S100A9* was most downregulated, and *MAGED4*, *C8orf30A*, and *IL-8* were the most upregulated in CE81T-1 cells. The expression of these four genes at the mRNA level was validated using the ESCC CE81T and KYSE cell lines and clarified in ESCC specimens using real-time polymerase chain reaction. Among 60 pairs of ESCC specimens (normal and tumor specimens), the expression level of *S100A9* mRNA was significantly lower in the tumor sections in comparison with the normal sections ($p = 0.0228$). In contrast, the expression level of *IL-8* mRNA was significantly higher in the tumor sections in comparison with the normal sections ($p = 0.0061$). Furthermore, *C8orf30A* expression was significantly correlated with ESCC metastatic status ($p = 0.0358$) and associated with poorer survival ($p = 0.036$), as determined by Kaplan-Meier analysis. Functional studies revealed that *S100A9* plays a suppressive role in the proliferation and migration of ESCC cells through the overexpression of ectopic *S100A9* and small interfering RNA, as determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)

* Corresponding author. Department of Microbiology & Immunology, College of Medicine, National Cheng Kung University, Tainan 701, Taiwan.
E-mail address: a713@mail.ncku.edu.tw (H.-S. Liu).

doi:10.1016/j.gmbhs.2012.04.002

and Transwell assays. Altogether, this study reveals that *C8orf30A* has the potential to be used as a novel biomarker for the prognosis for ESCC metastasis and survival. Furthermore, the *IL-8* and *S100A9* genes may have potential in ESCC diagnosis.

Introduction

Esophageal cancer (EC) is the eighth most common cancer in the world and is the ninth leading cause of cancer death in Taiwan.[1] EC is classified into two histological types: esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EA). These types show a variety of geographic distributions.[2] In Taiwan, there is an increasing trend of the incidence of ESCC, but not EA.[3] The prognosis of ESCC is very poor and the 5-year survival rate is $<$10%.[4] Accumulated evidence suggests that cyclin D1, p53, E-cadherin, and vascular endothelial growth factor (VEGF) can be used as independent predictors of the clinical outcome of ESCC patients. In this study, we identified ESCC migration-related genes by microarray analysis followed by real-time polymerase chain reaction (PCR) validation and clarified their roles in ESCC specimens.

Materials and methods

The human ESCC cell lines CE81T, CE81T-1, CE81T-2, KYSE150, KYSE510, KYSE70, and KYSE 170 were used in this study. Human esophageal tissue specimens were obtained from National Cheng Kung University Hospital (Tainan, Taiwan). All patients provided informed consent before the specimens were collected. Specific antibodies were used to detect *S100A9* expression by Western blotting. S100A9 protein expression in the ESCC specimens was detected using immunohistochemical (IHC) staining. The mRNA expression level of *S100A9*, *IL-8*, *MAGED4*, and *C8orf30A* were measured using real-time with specific polymerase chain reaction (PCR) primers. Migration of each ESCC cell line was investigated using wound-healing and transwell™ (Falcon, BD labware, Bedford, MA, USA) assays. The Transwell assay consisted of upper and lower chambers that were separated by a layer with an 8-μm pore size (Falcon, BD Labware, Bedford, MA, USA). Cells (7.0×10^5 cells/plate) were added to the upper chamber in 200 μL serum-free Dulbecco's Modified Eagle Medium (DMEM; Gibco BRL, Gaithersburg, MD, USA), and 600 μL DMEM containing 10% Fetal bovine serum (Gibco BRL, Gaithersburg, MD, USA) was added to the lower chamber as the chemoattractant. The cells were incubated at 37°C in a 5% CO_2 incubator overnight. Uninvaded cells were removed using a scraper, washed twice with phosphate buffered saline (PBS), and then fixed in 1% formaldehyde for 15 minutes at room temperature (RT). After fixation, the cells were washed twice with PBS and then stained with 0.1% crystal violet (Sigma-Aldrich, MD, USA) for 15 minutes at RT. Finally, the stained cells were washed with PBS and air dried. Three independent experiments were performed.

Results

The CE81T-1 cell subline was established from parental CE81T cells following Transwell screening in order to obtain cells with higher migration abilities. The CE81T-1 cells were more invasive than the CE81T cells. DNA microarray analysis was performed to identify the differentially expressed genes between these two cell lines. We found that *S100A9* was the most downregulated, whereas *MAGED4*, *C8orf30A*, and *IL-8* were most upregulated in CE81T-1 cells with strong migration activities.

Sixty pairwise ESCC specimens (tumor vs. normal), which consisted of specimens obtained from 54 male and six female patients, were analyzed in this study. Tumor status, stage, and the patient survival rate were evaluated to determine their correlation with mRNA deregulation of the *S100A9*, *MAGED4*, *C8orf30A*, and *IL-8* genes. Using the log-rank test, we found that the expression levels of *S100A9* and *C8orf30A* were negatively correlated with ESCC tumorigenesis. In contrast, *IL-8* was positively correlated with ESCC tumorigenesis. Furthermore, the *C8orf30A* expression level correlated with metastasis and the poorer survival rate of ESCC patients.

CE81T-2 was another subline established from CE81T cells by using the Transwell assay to screen for high-migration cells. The migration capability of the CE81T-2 cells was stronger than that of the CE81T and CE81T-1 cells. We also determined that the order of the migration capabilities of the four KYSE Japanese cell lines, from low to high, were KYSE150, KYSE510, KYSE70, and KYSE170, as shown by the results of the wound-healing analysis. The expression of *S100A9* was negatively correlated with the migration capability of the CE81T cells, which is consistent with the results of the microarray analysis (-1.92-fold). This inverse relationship was also observed in the KYSE cell lines. The mRNA expression levels of *MAGED4*, *C8orf30A*, and *S100A9*, in terms of pairwise comparisons with the CE81T-2 and CE81T cells as measured using real-time PCR, were consistent with the gene expression profiles of the CE81T-1 and CE81T cells determined using microarray analysis.

The ESCC cell lines were divided into two groups: the first group consisted of the CE81T and CE81T-2 cell lines and the second group consisted of the KYSE150, KYSE510, KYSE70, and KYSE170 cell lines. Our results show that the protein expression of *S100A9* in the CE81T-2, KYSE70, and KYSE170 cells with stronger migration capabilities was decreased when compared with their slow-migration counterparts. Taken together, both the mRNA and protein expression levels of *S100A9* were negatively correlated with the mobility of ESCC cells. In the same line of observations, the overexpression or knockdown of the *S100A9* gene in KYSE170 and KYSE150 cells, respectively, significantly changed the

migration and proliferation capabilities of these cells. Altogether, these results indicate that *S100A9* is negatively correlated with migration ability and cell proliferation.

Three ESCC tumor tissues from National Cheng Kung University hospital were utilized for the evaluation of *S100A9* expression by IHC staining. The protein expression of *S100A9* was significantly higher in well-differentiated tumors and adjacent tissues in comparison with poorly differentiated tissues.

Discussion and conclusion

In this study, differential gene expression profiles related to the mobility of the CE81T and CE81T-1 cell lines were revealed using DNA microarray analysis. Four genes—*MAGED4*, *C8orf30A*, *IL-8*, and *S100A9*—demonstrated deregulated mRNA expression in pairwise comparisons. To validate the microarray results, the gene expression levels of these four genes in the CE81T and KYSE cell lines with different migration capabilities were evaluated using real-time PCR. Our data show that *C8orf30A* and *MAGED4* gene expression levels were higher in CE81T-1 and CE81T-2 cells in comparison with the CE81T parental cells, indicating that the expression levels of these two genes are positively correlated with cell migration, which is consistent with the microarray results. The expression of *S100A9* was lower in CE81T-1 and -2 cells with stronger mobilities. The inverse relationship between *S100A9* expression and cell migration was also demonstrated in the four ESCC KYSE cell lines. Altogether, *S100A9* plays a suppressive role in ESCC cancer cell migration, further confirming the downregulation of *S100A9* that has been observed in other studies on ESCC.[5–7]

In contrast to *S100A9*, *IL-8* was identified as being overexpressed by the results of the DNA microarray. The differential gene expression profiles of CE81T-2 and CE81T were also screened using a customized microarray chip that contained 100 cancer-related genes. *IL-8* was consistently overexpressed in CE81T-2 cells with high-migration potential.

The deregulation of *S100A9* and *IL8* mRNA was further confirmed in clinical ESCC specimens using real-time PCR. Our data show that *IL-8* was significantly overexpressed, whereas *S100A9* was significantly underexpressed, in tumor sections in comparison with the adjacent normal tissues in 60 pairs of ESCC specimens. Because the the role of *S100A9* has never been explored in ESCC specimens, we further demonstrated the negative role of *S100A9* has on cell migration and proliferation by demonstrating the overexpression and silencing of *S100A9* expression. Using IHC staining, we also revealed that the S100A9 protein is highly expressed in well-differentiated tumors and adjacent normal tissues, but not in the poorly differentiated tissues. This result is consistent with the report by Luo et al.[6]

When comparing the clinical and pathogenic parameters of the ESCC specimens in terms of the deregulation of the four identified genes, *C8orf30A* was the only gene that demonstrated a significant correlation with metastatic status when we analyzed 53 ESCC patients ($p = 0.0358$). The expression of *C8orf30A* was also significantly correlated with the poorer survival rate of these patients ($p = 0.036$), suggesting that *C8orf30A* may be involved in metastasis and has potential use as a prognostic marker.

In summary, the *IL-8* and *S100A9* genes may have potential use in the diagnosis of ESCC. Moreover, *C8orf30A* has the potential to be used as a novel biomarker of ESCC metastasis and survival.

References

1. Lin JT. Endoscopic surveillance and treatment of esophageal cancer from high risk group: search and Discovery Research at ISU & EDA. 2009;1:4—8.
2. Toh Y, Oki E, Ohgaki K, et al. Alcohol drinking, cigarette smoking, and the development of squamous cell carcinoma of the esophagus: molecular mechanisms of carcinogenesis. *Int J Clin Oncol*. 2010;15:135—144.
3. Goan YG, Chang HC, Hsu HK, et al. An audit of surgical outcomes of esophageal squamous cell carcinoma. *Eur J Cardiothorac Surg*. 2007;31:536—544.
4. Wu IC, Wu DC, Huang CC, et al. Plasma decorin predicts the presence of esophageal squamous cell carcinoma. *Int J Cancer*. 2010;127:2138—2146.
5. Hermani A, De Servi B, Medunjanin S, et al. S100A8 and S100A9 activate MAP kinase and NF-kappaB signaling pathways and trigger translocation of RAGE in human prostate cancer cells. *Exp Cell Res*. 2006;312:184—197.
6. Luo A, Kong J, Hu G, et al. Discovery of Ca2+-relevant and differentiation-associated genes downregulated in esophageal squamous cell carcinoma using cDNA microarray. *Oncogene*. 2004;23:1291—1299.
7. Zhang J, Gao FL, Zhi HY, et al. Expression patterns of esophageal cancer deregulated genes in C57BL/6J mouse embryogenesis. *World J Gastroenterol*. 2004;10:1088—1092.